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Detection of Adenovirus DNA in Peripheral Blood Mononuclear Cells by Polymerase Chain Reaction Assay

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Adenovirus can establish persistent infections which may reactivate and cause disease in immunocompromised hosts. Lymphocytes have been postulated to serve as a site of adenoviral persistence based upon the ability to isolate adenovirus from tonsils and to detect adenovirus DNA by Southern blot hybridization in peripheral blood mononuclear cells (PBMC). To test this hypothesis, a more sensitive and specific polymerase chain reaction (PCR) assay was developed to detect adenovirus DNA. Two sets of nested primers were designed to conserved sequences in the adenovirus E1A and hexon genes. The E1A and hexon primers amplified DNA from representative adenoviral serotypes in all six adenoviral groups (A–F). Both primers detected a single copy of the adenovirus type 2 genome but were less sensitive for the group B type 35. None of 33 PBMC specimens from healthy adults and only one of 40 pediatric samples was positive (at a low level) for adenovirus DNA by nested PCR assay. In comparison, PBMC from two children with fatal adenoviral infection were both strongly positive for adenovirus DNA. It is concluded that, in contrast to a previous study, PBMC are not a common site of persistent group C adenoviral infection. In addition, assay of PBMC by the adenovirus-specific PCR may help detect early invasive disease and warrants further evaluation. ***J. Med. Virol. 51: 182–188, 1997.***

KEY WORDS: adenoviral infection; group C Ad2; group B Ad35; viral persistence; lymphocytes; nested PCR assay

INTRODUCTION

Adenovirus, a common childhood pathogen, can cause serious disease in immunocompromised hosts, including pneumonia, hepatitis, nephritis, and encephalitis [Horwitz, 1990; Hierholzer, 1992]. Adenovirus was documented to cause hepatitis in 2% of pediatric liver transplant recipients [Michaels et al., 1992], and nephritis has been reported in renal transplant patients [Stadler et al., 1977]. Adenoviral disease was reported in 1% of bone marrow transplant recipients with unmodified grafts [Shields et al., 1985]. More recently, an increased incidence of adenoviral infection (21%) and disease (7%) was documented in bone marrow transplant recipients, most of whom received T cell-depleted grafts [Flomenberg et al., 1994]. In this study, adenoviral disease was associated with a mortality of 50%. The most common serotypes were the group C Ad1, Ad2, and Ad5 and the group B Ad35, but the sources of the infections were not identified. In healthy individuals, there is evidence that adenovirus can establish persistent infections; e.g., adenovirus may be shed in the feces for months to years after acute infection [Fox et al., 1977]. Therefore, it is likely that infections in immunocompromised hosts are in part due to reactivation of persistent adenovirus.

The reservoirs of adenoviral infection have not been well defined, and the mechanism of adenoviral persistence is unknown. These issues are important not only for understanding the pathogenesis of adenoviral disease but also in the evaluation of the safety and efficacy of adenoviral vectors for gene therapy. Adenovirus offers unique advantages as a gene therapy vector, but recent data using a mouse model suggest that vector-

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transduced cells are rapidly destroyed by adenovirus-specific cellular immune responses [Yang et al., 1994]. The presence of endogenous virus and memory antiviral immune responses may pose additional problems for adenovirus-mediated gene therapy in humans. Conversely, the potential of adenoviral vectors to persist in some human cell types needs to be evaluated. For instance, in a study using an E1-region-deleted adenoviral vector to transduce bone marrow cells, virus could be recovered from the bone marrow cultures 2 months postinfection [Mitani et al., 1994].

Lymphocytes have been implicated as a reservoir based upon the ability to isolate adenovirus from tonsils and adenoids of asymptomatic individuals [Van Der Veen and Lambriex, 1973]. However, the cell type was not identified, and the presence of a low level productive infection in tonsillar fibroblasts rather than lymphocytes cannot be excluded. In one study, adenovirus DNA was detected in peripheral blood mononuclear cells (PBMC) from 13 of 17 healthy adults by Southern blot hybridization [Horvath et al., 1986]. The authors identified lymphocytes as the site of adenoviral infection by in situ DNA hybridization. In contrast to its ability to cause a lytic infection in epithelial and fibroblast cell lines in vitro, adenovirus inefficiently infects PBMC and most lymphocytes cell lines and does not cause obvious cytopathic effects. However, the ability of adenovirus to replicate in a minority of B- and T-cell lines was documented in one short-term study [Lavery et al., 1987]. In long-term studies, experimental adenoviral infection was shown to establish low-level persistence in lymphocyte and monocyte cell lines [Andiman and Miller, 1982; Chu et al., 1992]. Furthermore, a spontaneous, chronic adenoviral infection was documented in an Epstein Barr virus-associated B-cell lymphoma from a bone marrow transplant recipient [Flomenberg et al., 1996]. A cell line derived from this lymphoma maintained a homogeneous, highly productive adenoviral infection for up to 6 months, which indicates that adenovirus can replicate in lymphocytes without inducing cell lysis under some circumstances.

In order to identify the sites of persistent adenoviral infection, a highly sensitive and specific polymerase chain reaction (PCR) assay for the detection of adenovirus DNA was developed. Two adenovirus-specific nested primer sets, designed to conserved sequences in the adenovirus genes encoding the E1A and hexon proteins, were shown to amplify the DNA of representative serotypes from all six adenoviral groups, A–F. As a first step toward identification of the sites of adenoviral persistence, PBMC specimens from 73 healthy children and adults were tested for the presence of adenovirus DNA by nested PCR assay. For a comparison, PBMC samples from two patients with fatal adenoviral disease were also tested with the adenovirus-specific PCR assay.

MATERIALS AND METHODS

Study Participants

Heparinized blood specimens (5 ml) were obtained from 33 healthy adult volunteers. Blood (2 ml) from 40 children hospitalized for minor surgical procedures and two children with documented adenoviral disease was obtained from the clinical pathology laboratory.

Viruses and Cell Lines

The Ad2 and Ad35 prototypes were obtained from M. Horwitz (Albert Einstein College of Medicine, Bronx, NY). Crude lysates of the Ad1, Ad3, Ad4, Ad8, Ad11, Ad12, Ad31, Ad37, and Ad40 prototypes were provided by D. Erdman (Centers for Disease Control and Prevention, Atlanta, GA). Ad2 and Ad35 were propagated in the lung epithelial cell line A549 (CCL 185; American Type Culture Collection, Rockville, MD).

Preparation of DNA Samples

PBMC from 2–5 ml of heparinized blood were isolated by Ficoll-Hypaque density gradient centrifugation, resuspended in 10 ml RPMI 1640 (GIBCO, Gaithersburg, MD), and washed twice with phosphate-buffered saline (PBS). Cells (5×10^5) were lysed in 100 μl PCR buffer (10 mM Tris [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, 0.5% Tween 20). After incubation with 10μg (0.01%) Proteinase K (Boehringer-Mannheim, Indianapolis, IN) for 45 min at 56°C, lysates were heat-inactivated for 10 min at 95°C and stored at –20°C until use.

Purified adenovirus DNA was prepared from Ad2- and Ad35-infected A549 cells by a modified Hirt procedure, as previously described [Horwitz et al., 1976]. Based on an MW of 23.1×10^6 g, it was estimated that one adenovirus genome represents 0.04 fg of DNA.

PCR Primers

Nested adenovirus-specific primers were designed to conserved sequences in the adenovirus E1A and hexon genes based upon all available sequences from different adenoviral serotypes. The software program Oligo (NBI, Plymouth, MN) was utilized to help optimize the design of the PCR primers, and customized primers were purchased from NBI. The sequences of the E1A and hexon (HEX) primers and locations within the adenovirus genes are listed in Table I.

Adenovirus-Specific PCR Assay

DNA from each PBMC sample was denatured at 95°C for 10 min and cooled on ice for 10 min immediately before use. Ten Microlicers of PBMC or purified adenovirus DNA was added to 90 μl of a reaction mixture containing Taq buffer (10 mM Tris [pH 8.3], 50 mM KCL, 0.01% gelatin), 0.2 mM dNTP (Pharmacia, Piscataway, NJ), 2.5 U Taq DNA polymerase (Promega, Madison, WI), and either 0.4 μM E1A 1,2 outer primers plus 3 mM $MgCl_2$ or 0.2 μM HEX 1,2 outer primers plus 1.5 mM $MgCl_2$. The following thermal cycler regimen was used: one cycle at 95°C × 2 min, 55°C (E1A) or 58°C (HEX) × 1 min, and 72°C × 1 min; 35 cycles at 94°C × 1 min, 55°C (E1A) or 58°C (HEX) × 1 min, and 72°C × 1 min; then 72°C × 7 min.

Nested PCR assays using the E1A and hexon inner primers were undertaken on 2 μl aliquots from the above PCR reactions. Reactions were carried out in a

TABLE I. Sequence and Locations of the Adenovirus E1A and Hexon Nested Primer Sets

Adenovirus primers	Oligonucleotide sequences[a] (5′ to 3′)	Location on genome	PCR product (bp)
		E1A region:[b]	
E1A 1 (sense)	GAGTGAACTTTGACCGTYTACGTG (24-mer)	366–389	
E1A 2 (anti-sense)	TCCACCTACAAATCATACAGWTCGT (25-mer)	736–712	371
E1A 3 (sense)	TCCGCGTACCGTGTCAAAGT (20-mer)	423–442	
E1A 4 (anti-sense)	GGAACGCGAAGGTGTCTCATT (20-mer)	594–575	172
		Hexon gene:[c]	
HEX 1 (sense)	CGATGATGCCGCAGTGGTCTTA (22-mer)	14–35	
HEX 2 (anti-sense)	GCACGCCGCGGATGTCAAAGTA (22-mer)	319–298	306
HEX 3 (sense)	GCGCCACCGAGACGTACTTCA (21-mer)	98–118	
HEX 4 (anti-sense)	CGGTATCCTCGCGGTCCACAG (21-mer)	229–209	132

[a]Y = C + T. W = A + T.
[b]Location of the E1A primers based on the Ad7 sequence; numbering starts at the right end of the genome.
[c]Location of the hexon primers based on the Ad2 sequence; numbering starts at the beginning of the hexon open reading frame.

final volume of 100 μl containing Taq buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP, and 0.2 μM of either the E1A or HEX 3,4 primers. Amplification was performed with 35 cycles at 94°C × 1 min, 52°C (E1A) or 58°C (HEX) × 1 min, and 72°C × 1 min; then 72°C × 7 min. Aliquots (10 μl) of each PCR reaction were run on a 1.5 or 3% NuSieve agarose gel (FMC, Rockland, ME) along with a 100 bp ladder (Pharmacia), and DNA bands were stained with ethidium bromide.

Strict precautions were followed to prevent contamination of the PCR assay. PBMC samples were processed in a separate laboratory. PCR reactions were prepared in a laminar flow hood decontaminated with UV light for 20 min prior to use. Filtered pipet tips and individually aliquoted reagents were utilized and gloves changed frequently during the preparation of PCR mixtures. All PCR assays were carried our with appropriate negative and positive controls.

RESULTS

Adenovirus-Specific Primers Amplify DNA From All Adenoviral Groups

Although all adenoviral serotypes share a similar structure and genomic organization and the DNA sequences among serotypes within each group are generally well conserved, there is substantial sequence heterogeneity among serotypes in different groups [Horwitz, 1990]. For instance, the overall intragroup homology of serotypes from adenoviral groups B, C, and D ranges from 80% to 99% by DNA hybrization analysis, whereas the homology between groups is less than 20% [Green et al., 1979]. Therefore, primers were based on conserved DNA sequences among serotypes from different groups in order to optimize the cross-reactivity between serotypes.

One set of adenovirus-specific primers was designed to amplify a portion of the adenovirus gene that encodes E1A. The E1A region, located from 0 to 4 map units on the adenovirus genome, was selected because it represents a trans-activating region which controls viral transcription, a crucial function conserved between serotypes. A comparison between available E1A sequences from the group A Ad12, group B Ad7, and group C Ad2 and Ad5 revealed an overall homology of approximately 50% between groups [Van Ormondt et al., 1980]. Primers were designed based on E1A regions that exhibited above average homology among the different serotypes. In order to further optimize the cross-reactivity of the E1A primers, one degenerate nucleotide was used in each primer.

The hexon gene, which is located between 50 and 60 map units and encodes the major outer capsid protein, was selected for the second primer set. There is evidence that the N-terminal sequence is well conserved between serotypes [Toogood et al., 1989]. This is supported by X-ray crystallographic data which indicate that the hexon N-terminus is crucial for intermolecular interactions between hexon molecules [Roberts et al., 1986]. A comparison of hexon sequences from the group C Ad2 and Ad5 [Kinloch et al., 1984] and the group F Ad40 and Ad41 [Toogood et al., 1989] revealed highly conserved regions (homology greater than 95%) in the N-terminus, based upon which the HEX primers were designed. Hexon sequences were not available for other adenoviral groups at the time that this study was initiated.

The E1A 1,2 and HEX 1,2 outer primers were evaluated for the ability to amplify DNA from different adenoviral serotypes. Two representative serotypes from each adenoviral group A–F were tested. Both primer sets amplified appropriate-sized DNA fragments from crude lysates of cells infected with each serotype (Fig. 1). There was some variation in the size of the E1A bands among different serotypes, which likely reflects differences in the lengths of the intervening sequences between primers. For instance, the predicted size of the E1A fragment amplified from the group A Ad12 is 335 bp, the group B Ad7 fragment 371 bp, and the group C Ad2 product 354 bp. Subsequent analysis of E1A sequences for the group E Ad4 and group F Ad40 (Genbank accession numbers M14918 and M38595) predicted PCR products of 371 and 347 bp, respectively. E1A sequences were not available for the other serotypes tested. Doublets were amplified by the E1A primers from the group D Ad8 and group F Ad40 specimens only and were not further investigated for the purposes of this study. The lower 300 bp band present in these two lanes may represent mispriming, with either viral

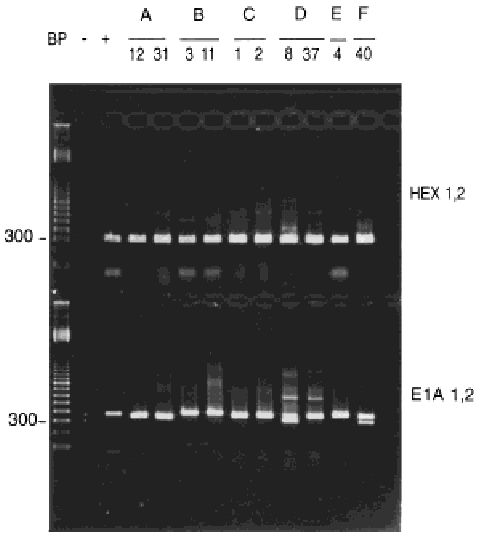

Fig. 1. Detection of representative adenoviral serotypes from groups A–F by the adenovirus-specific PCR assay. Adenovirus DNA (approx. 300 bp fragment) was amplified from 10 μl crude lysates of cells infected with each serotype using the HEX 1,2 primers (top). The E1A 1,2 primers (bottom) amplified approx. 400 bp products from each serotype. BP, 100 bp marker; –, no DNA template; +, purified Ad2 DNA (10^3 genomes).

sequences or cellular DNA present in the crude cell lysates (as suggested below).

Primers Vary in Sensitivity for Groups B and C Serotypes

Although both the HEX and E1A primers successfully amplified representative serotypes from all six adenoviral groups, it was postulated that the sensitivity of the PCR assay for serotypes from different groups would vary. To test this hypothesis, the ability of the adenoviral primers to amplify limiting amounts of DNA from the group C Ad2 and the distantly related group B Ad35 was compared. Both E1A 1,2 and HEX 1,2 primers were based on known Ad2 sequences, but neither the Ad35 E1A nor the hexon sequence was available for analysis. Serial dilutions of purified Ad2 and Ad35 DNA were tested with both sets of outer primers (Fig. 2). The E1A 1,2 primers were 10- to 100-fold less sensitive for Ad35 DNA (limit of detection 10^3–10^4 genomes) compared to Ad2 DNA (100 genomes). Furthermore, the HEX 1,2 primers were 1,000-fold less sensitive for Ad35 (limit of detection 10^5 genomes) compared to Ad2 (100 genomes) (data not shown). Therefore, although HEX and E1A primers were based on sequences within the E1A and hexon genes that are conserved among several serotypes from different groups, these results suggest that the group B Ad35 has significant sequence heterogeneity in these regions.

Adenovirus-Specific Nested Primers Detect One Copy of the Ad2 Genome

In order to increase the sensitivity of the adenovirus-specific PCR assay, E1A and HEX inner primers were designed for use in a nested PCR assay. Purified Ad2 DNA was titrated in the nested PCR assay in order to measure its sensitivity. As shown in Figure 3, both sets of nested primers (E1A 3,4 and HEX 3,4) detected one genomic copy of Ad2 DNA.

Adenovirus DNA Is Present in PBMC From Patients With Adenoviral Disease

PBMC from two patients with fatal adenoviral disease were strongly positive for adenovirus DNA by the adenovirus-specific PCR assay (Fig. 4). Patient 1 was a neonate who developed a fatal adenoviral pneumonia, documented by a positive nasopharynx culture and intranuclear inclusions in the lung at autopsy. The isolate was not available for serotyping. The PBMC sample from patient 1 was positive for adenovirus DNA using the E1A outer primers but not the HEX outer primers. The presence of adenovirus DNA was confirmed by the E1A inner primers, and, as with the HEX outer primers, the HEX inner primers were negative (data not shown). Patient 2 was a 3-year-old bone marrow transplant recipient who developed fatal Ad2 pneumonia and hepatitis, documented by a positive culture from lung tissue and intranuclear inclusions in the lung and liver at autopsy. Adenovirus DNA was amplified from the PBMC of patient 2 by both the HEX and E1A outer primers. The positive results were confirmed by nested PCR with both the HEX and E1A inner primers (data not shown). As a control, neither the HEX nor E1A outer primers amplified adenovirus DNA from PBMC from a healthy adult. However, the E1A primers amplified a 300 bp band from each PBMC sample, which was not amplified from the purified adenovirus DNA. This product was also amplified from all other PBMC samples from healthy controls, three cord blood samples, and three human cell lines (data not shown), which suggests that it represents mispriming of the E1A primers with an unknown cellular sequence. No band was amplified from any of these cell samples using the E1A inner primers, which confirmed that the 300 bp band was nonspecific. As another control, in order to document that the PBMC samples did not nonspecifically inhibit the PCR reaction, purified Ad2 DNA mixed with control PBMC DNA was successfully amplified by both primers.

Adenovirus DNA Is Not Detectable in PBMC From Normal Controls

In order to determine whether PBMC are a reservoir of persistent adenoviral infection, 73 samples from healthy children and adults were tested with the adenovirus-specific nested PCR assay. None of 40 PBMC samples from adult individuals were positive with either the E1A or HEX nested primers. Only one of 33 PBMC specimens from children was positive: a low level of adenovirus DNA was amplified from one sample with the HEX inner primers only (data not shown).

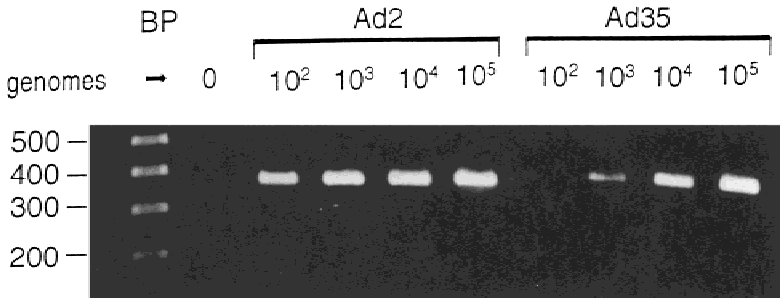

Fig. 2. Comparison of the sensitivity of the E1A primers for Ad2 and Ad35 DNA templates. Serial 10-fold dilutions of purified Ad2 and Ad35 DNA were tested by PCR assay using the E1A 1,2 primers BP, 100 bp marker; 0, no DNA template.

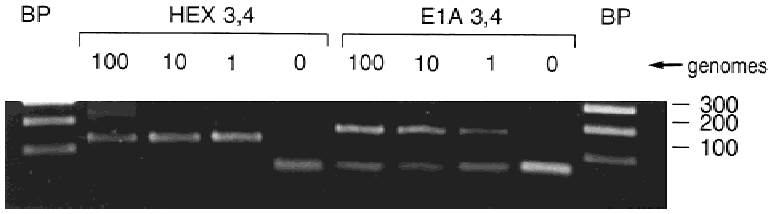

Fig. 3. Titration of Ad2 DNA in nested PCR assay. Serial 10-fold dilutions of purified Ad2 DNA were tested by PCR assay using both HEX and E1A nested primers. The HEX 3,4 primers amplified a 130 bp product. The E1A 3,4 primers amplified a 170 bp product. 0, negative control without DNA. Note that the low MW bands present in the lanes without DNA likely represent primer-dimers.

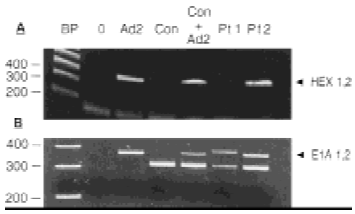

Fig. 4. Detection of adenovirus DNA in peripheral blood mononuclear cells (PBMC) from patients with severe adenoviral disease. PBMC DNA from patients (Pt) 1 and 2 were tested by PCR assay with the HEX 1,2 primers (**A**) and the E1A 1,2, primers (**B**). BP, 100 bp marker; 0, no DNA; Ad2, purified DNA (10^3 genomes); Con, control PBMC DNA from a healthy volunteer; Con + Ad2, control PBMC DNA spiked with 10^3 genomes of purified Ad2 DNA.

DISCUSSION

We developed a highly sensitive and specific PCR assay for the detection of adenovirus DNA in order to identify the reservoirs and prevalence of persistent infection in humans. There is clinical evidence for persistence of adenovirus in vivo, and reactivation of latent virus may be an important source of adenoviral disease in immunocompromised patients. In addition, the presence of endogenous adenovirus may interfere with the efficacy of adenovirus vector-mediated gene therapy. Two sets of nested primers were designed from conserved regions within the adenovirus E1A and hexon genes, which are located on opposite halves of the viral genome. Both adenovirus-specific primer sets amplified successfully DNA from serotypes from all six adenoviral groups but were most sensitive for the common group C adenoviruses.

Since lymphocytes have been postulated to be a major site of persistent adenoviral infection, PBMC from healthy individuals were tested for the presence of viral DNA with the adenovirus-specific PCR assay. Adenovirus DNA was detected by nested PCR in only one of 73 samples from nonimmunocompromised children and adults. There was no evidence for nonspecific inhibition of the PCR assay, as demonstrated by mixing experiments with PBMC DNA and purified adenovirus DNA. In addition, these negative results suggest that there was no problem with contamination or lack of specificity of the PCR assay. These data do not exclude the presence of adenovirus DNA in PBMC at concentrations below the limit of sensitivity of the PCR assay (one copy per 5×10^4 cells). In particular, low levels of non-group C adenovirus DNA could be missed because the sensitivity of the PCR assay is reduced for non-group C serotypes. However, the group C adenoviral serotypes are the most common serotypes found in epidemiologic studies [Schmitz et al., 1983] and would most likely establish persistent infections in vivo.

In contrast to the lack of detection of adenovirus DNA in PBMC from healthy individuals, the adenovirus-specific PCR assay readily amplified virus from specimens from two patients with fatal adenoviral disease. PBMC from a bone marrow transplant patient with Ad2 pneumonia were positive for adenovirus DNA with both the hexon and E1A outer primers, and the positive results were confirmed by nested PCR with the hexon and E1A inner primers. In addition, PBMC from a neonate with adenoviral pneumonia, whose isolate was not available for serotyping, were positive by PCR with the E1A outer primers but not the hexon primers, which was confirmed by nested PCR with the E1A inner primers. The fact that adenovirus DNA was amplified with the E1A primers but not the hexon primers

suggests that the adenoviral infection in the neonate was caused by a non-group C serotype. For example, the group B Ad3 or Ad7 are likely candidates to cause severe pneumonia in a neonate and would be poorly amplified by the hexon primers based on our data with the related group B Ad35. These data suggest that invasive adenoviral disease may be associated with viremia despite the fact that adenovirus is rarely cultured from blood specimens. Further study is needed to determine the cell type(s) in PBMC infected with adenovirus and the level of viral protein expression. It remains possible, but unlikely, that the presence of viral DNA in PBMC from the two patients with adenoviral disease, but not healthy individuals, may simply correlate with an immunocompromised state. To address this issue, we have begun to test PBMC from bone marrow transplant recipients without evidence of adenoviral infection. Samples from all 10 patients tested so far were negative by PCR assay with both hexon and E1A outer primers (personal observation, P. Flomenberg).

The adenovirus-specific PCR assay may prove useful for the early detection of adenoviral disease, analogous to the use of the PCR assay on blood specimens to identify patients at high risk for cytomegalovirus (CMV) disease [Patel et al., 1995]. Like CMV, which may be shed asymptomatically in urine and saliva, adenovirus may be cultured from stool and urine in patients without symptoms. Diagnosis of adenoviral disease in immunocompromised patients usually requires tissue biopsy for culture and pathology. Although there is presently no available therapy for adenovirus, we anticipate that effective antivirals will be developed in the future. For instance, the new broad-spectrum antiviral drug cidofovir has been shown to be active against human adenoviral infection in a rabbit ocular model [Deoliveira et al., 1996]. Therefore, we conclude that a noninvasive assay for the early detection of adenoviral disease may be helpful and that further testing of the adenovirus-specific PCR assay on blood samples from immunocompromised patients with and without adenoviral disease is warranted.

It was found, however, that the sensitivity of the adenovirus-specific PCR assay using the E1A and HEX primers is limited by the sequence heterogeneity among different adenoviral types. Adenovirus-specific PCR assays have been developed by other investigators for the purpose of testing clinical samples [Allard et al., 1990; Hierholzer et al., 1993]. Our data on the ability of the hexon primers to detect serotypes from all six adenoviral groups is in agreement with those of Allard et al. [1990], who used hexon primer sequences which overlap those independently designed by us. However, we have identified substantial decreased sensitivity of the hexon primers for group B serotypes; i.e., group B Ad35 DNA was amplified 10^3-fold less well than Ad2 DNA. We have analyzed the group B Ad3 and Ad7 hexon sequences (GenBank accession numbers X76549 and X76551), subsequently made available by P. Pring-Akerblom and T. Adrian (Hannover, Germany), after our study began. Consistent with the low sensitivity of the HEX primers for Ad35, the related Ad3 and Ad7 hexon genes contain significant sequence differences compared with the Ad2 hexon at the N-terminus, including multiple mismatches with the HEX primers. In the present study, nested primers to the E1A region increased the sensitivity of the PCR assay for Ad35 DNA over 10-fold, but the E1A primers still amplified Ad35 DNA less well than Ad2 DNA. Therefore, for the optimal design of adenovirus PCR primers that are sensitive for all serotypes, we recommend that DNA sequence information be obtained on multiple serotypes from all groups. For instance, in a recent study, adenoviral primers based on virus-associated (VA) RNA sequences from multiple serotypes amplified DNA from representative serotypes from all groups, as well as most clinical isolates tested [Kidd et al., 1996]. However, the limit of detection of the primers for different serotypes was not addressed. In particular, for single-copy detection of group B serotypes, which represent important pathogens in immunocompromised hosts, the use of group B- or serotype-specific primers may be required.

From this study we can conclude that PBMC are not a common reservoir of group C adenoviral infection. This result contradicts a previous study by Horvath et al. [1986], which documented adenoviral group C DNA in both PBMC and human lymphoid cell lines. The authors detected the presence of adenovirus DNA in PBMC samples from a majority of healthy adults (76%) by Southern blot assay using a radiolabeled mixture of cloned Ad2 HindIII fragments as a probe. In positive samples, viral DNA was identified in approximately 1–2% of cells by in situ DNA hybridization. As shown by others, however, DNA hybridization may generate false-positive results due to the presence of homologous cellular sequences which cross-hybridize with adenovirus DNA [Arrand et al., 1983]. This explanation is supported by another study which did not detect adenovirus DNA in lymphoid cell lines using stringent DNA hybridization conditions [Lavery et al., 1987]. The adenovirus-specific PCR assay is more sensitive and specific than DNA hybridization techniques and should prove useful for evaluating further the sites and prevalence of persistent adenoviral infection in humans.

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